The independent claims have been amended to clarify that the nucleic acid sequence used to transform the plant encodes an enzyme capable of modifying the utilization of an intermediate substrate in a secondary metabolic pathway associated with a nutritional profile of a plant. Support for this amendment is found in the specification at e.g., the paragraph bridging pages 8 and 9, wherein it is explained that substrates of interest are particularly those compounds within one to five biochemical steps of final product formation – i.e., intermediate substrates.

The independent claims have also been amended to recite that the enzyme is not naturally occurring in the second metabolic pathway. That is, the nucleic acid sequence introduced into the plant encodes an enzyme that itself is not part of the secondary metabolic pathway of interest. Support for this amendment is found throughout the specification, e.g., in Examples 1-6 at pages 61-76, which describe the use of a nucleic acid sequence encoding choline oxidase to modify the utilization of an intermediate substrate in the phenylpropanoid secondary metabolic pathway, thereby reducing sinapine levels. As shown in Figure 2, choline oxidase is not part of the phenylpropanoid pathway. Broad support is also found at page 31, lines 10-12 wherein it is explained that the term "heterologous", in the context of the sugar alcohol secondary metabolic pathway, refers to an enzyme not normally associated with phytate biosynthesis in a plant cell.

The independent claims have also been amended to specify that the nucleic acid sequence encoding an enzyme capable of modifying the utilization of an intermediate substrate in a secondary metabolic pathway is operably linked to a seed-specific promoter. This amendment finds support in e.g,. the claims of record and Examples 1-18, which involve a use of a seed-specific promoter.

The independent claims have been further amended to specify that the plant species is selected from the group consisting of corn, canola, wheat,

barley, oats, alfalfa, soya beans and sorghum. Support for this amendment is found e.g., at page 31, lines 12-16.

Independent claim 82 additionally specifies that the secondary metabolic pathway is the phenylpropanoid pathway. Support for this amendment is found, e.g., in the original claims of record and examples 1-9.

Independent claim 83 specifies that the enzyme encoded by the nucleic acid sequence operably linked to a seed-specific promoter is a choline-metabolizing enzyme. Support for this amendment is found, e.g., in the original claims and at pages 18-26 of the specification.

The dependent claims all concern subject matter recited in the previous claim set and find support therein.

In view of the foregoing, Applicants respectfully submit that the amendments do not constitute new matter.

Rejection under 35 USC § 112

Claims 34-38, 40-46 and 77-80 of record stand rejected under 35 USC § 112, first paragraph on the grounds that the new limitation "with the proviso that said plant is not rice or *Arabidopsis*" constitutes new matter. The presently amended claims do not contain this proviso, therefore the rejection is rendered moot.

Claims 34-38, 40-46 and 48-80 of record stand rejected under 35 USC 112, first paragraph, on the grounds that the expression "non-native to said secondary metabolic pathway" or "non-native to said phenylpropanoid pathway" constitutes new matter. This rejection is obviated by the present amendment of the claims to recite an enzyme that is "not naturally occurring" in the secondary metabolic pathway. Applicants believe that this amendment is fully supported in the description as discussed above, and clarifies that the nucleic acid molecule introduced into the plant encodes an enzyme that does not have the same enzymatic activity of an enzyme already present in the secondary metabolic pathway of interest in the plant.

The rejections of claims 34-38, 40-46 and 48-80 under 35 USC § 112, second paragraph, as being indefinite for the inclusion of the expression "non-native" is also obviated by the present amendment. Applicants respectfully submit that the expression "not naturally occurring in said secondary metabolic pathway" would be readily understood by the skilled person in the context of the instant application.

The rejection of claim 62 of record under 35 USC § 112, second paragraph is obviated by the cancellation of this claim.

Concerning 35 USC § 102

The Examiner has rejected various claims as lacking novelty over the following references:

U.S. Patent No. 5,948,667 (Cheng *et al.*);

International Publication No. WO 97/23599 (Chapple *et al.*); and

International Publication No. WO 93/05160 (Van Doorsselaere *et al.*)

Applicants respectfully submit that the presently amended claims are patentably distinguished from each of the above references for at least the following reasons.

Cheng *et al.*

Cheng *et al.* teach methods nucleic acid molecules encoding xylanase and methods of producing xylanase. Cheng *et al.*

The Examiner states that the methods taught by Cheng *et al.* comprise the transformation of *B. napus* with an expression vector comprising a seed-specific promoter (the oleosin promoter) and a coding sequence for xylanase. The transformation of the plant with a coding sequence for xylanase results in the production of transgenetic plants with altered nutritional profiles because they contain a higher level of xylanase than wild-type plants.

The Examiner further states that Applicants' arguments filed in the preceding Amendment were not persuasive. In that Amendment, Applicants

argued that the xylanase enzyme described by Cheng *et al.* is described to act upon xylan components in hemicellulose, a final product found in plant primary and secondary cell walls. In response, the Examiner stated that hemicellulose is a substrate in plant secondary pathways which involve metabolism (i.e., via hydrolysis or other degradation or modification).

Applicants respectfully submit that the presently amended claims patentably distinguish from Cheng *et al.* in that Cheng *et al.* do not teach or suggest the limitations reciting "selecting a nucleic acid sequence for its ability to encode an enzyme capable of modifying the utilization of an intermediate substrate in a secondary metabolic pathway associated with a nutritional profile of a plant; said enzyme not naturally occurring in said secondary metabolic pathway."

More specifically in this regard, Applicants respectfully submit that Cheng *et al.* fails to teach that the enzymatic activity of recombinant xylanase acts upon any intermediate substrate in a secondary metabolic pathway associated with a nutritional profile of a plant. While the Examiner asserts that hemicellulose is a substrate in plant secondary pathways which involve metabolism, Applicants contend that Cheng *et al.* does NOT teach that: 1) the xylanase Cheng *et al.* introduced into plants acts upon hemicellulose in any secondary metabolic pathway of a plant; and 2) the xylanase enzyme is not naturally occurring in whatever secondary pathway [not yet identified by the Examiner] might involve metabolism.

Applicants respectfully submit that Cheng *et al.* instead teaches an oleosin-xylanase fusion wherein the xylanase is rendered immobilized in the seed oil-body membrane of canola meal. (see e.g., Cheng *et al.*, col. 11, lines 14-24 and col. 18, lines 16-67). Applicants thus submit that the xylanase taught by Cheng *et al.* is available to act upon hemicellulose only after the plant is harvested and the oil extracted (Cheng *et al.*, col 11, lines 14-17). As an immobilized enzyme, the xylanase taught by Cheng clearly would be

unavailable for modifying an intermediate substrate in a secondary metabolic pathway of a plant.

Since Cheng *et al.* does not teach each and every limitation of the subject claims, Applicants therefore respectfully request that the rejections over Cheng *et al.* be withdrawn. If the Examiner persists in the rejection, Applicants respectfully request that the Examiner identify the specific teaching in Cheng *et al.* which describes the aforementioned claim recitation.

Chapple *et al.*

The Examiner states that:

> Chapple *et al.* teach the transformation of plants with the F5H gene in order to alter the lignin content in plants. Chapple *et al.* exemplify this method in the transformation of Arabidopsis thaliana (a crucifer) and further teach that this method is useful to transform other plants such as alfalfa, rice, maize and oil seed rape (Brassica)(p. 7, lines 15-20). Chapple *et al.* teach the growth of such plants to permit the formation of seed, and the recovery of said seed (p. 19, lines 4-5). Chapple *et al.* teach the use of tissue specific promoters (p. 15, lines 25-29). Chapple *et al.* teach method steps in which at least one genetically altered plant having altered lignin content is identified (p. 24 line 25-p. 24 line 7, Tables 1 and 2). Since the F5H gene effects the production of a produce in the phenylpropanoid pathway which is necessary for the production of sinapine, (i.e. 5-hydroxyferulic acid) plants with decreased F5H activity as taught by Chapple *et al.* would inherently have the property of decreased sinapine levels compared to the wild type plants.

Applicants respectfully submit that the presently amended claims patentably distinguish from Chapple *et al.* Chapple *et al.* transformed *Arabidopsis* plants with a gene encoding the F5H enzyme to reduce lignin synthesis. As shown in Figure 1 of Chapple *et al.*, the F5H enzyme naturally occurs (i.e. is found in) the plant metabolic pathway for lignin synthesis.

The presently amended claims specify that the enzyme does not naturally occur in the secondary metabolic pathway. Chapple *et al.* do not teach or suggest transforming a plant with nucleic acid molecule encoding an enzyme that does not naturally occur in the secondary metabolic pathway in which the utilization of an intermediate substrate is to be modified, as presently claimed.

Van Doorsselaere *et al.*

The Examiner states that:

Van Doorsselaere *et al.* teach the transformation of plants with a nucleic acid encoding O-methyl transferase (OMT) in order to alter the lignin content in plants. Van Doorsselaere *et al.* exemplify this method in the transformation of poplar trees and further teach that this method is useful to transform other plants such as alfalfa, rice, maize and oil seed rape (Brassica)(p. 13, lines 15-26). Van Doorsselaere *et al.* teach method steps in which at least one genetically altered plant having altered lignin content is identified (p. 21-23). Since the OMT gene effects the production of a product in the phenylpropanoid pathway which is necessary for the production of sinapine, (i.e. ferulic acid) plants with decreased OMT activity as taught by Van Doorsselaere *et al.* would inherently have the property of decreased sinapine levels compared to the wild type plants.

Applicants respectfully submit that the claims, as presently amended, patentably distinguish from Van Doorsselaere *et al.* The presently amended claims patentably distinguish from Van Doorsselaere *et al.* for the same reasons that they patentably distinguish from Chapple *et al.* — i.e. Van Doorsselaere *et al.* do not teach transforming a plant cell with an enzyme capable of modifying the utilization of an intermediate substrate in a secondary metabolic pathway associated with a nutritional profile of a plant, wherein the enzyme does not naturally occur in the secondary metabolic pathway.

Van Doorsselaere *et al.* describe a strategy for modifying lignin content and composition in plants by inserting into the plant genome by transformation one or more additional copies of the O-methyl-transferase gene that is an essential enzyme in the metabolic pathway for the biosynthesis of lignin monomers, which is part of the phenylpropanoid secondary metabolic pathway. Thus, Van Doorsselaere *et al.'s* strategy is to modulate the level of expression of an enzyme that naturally occurs in the secondary metabolic pathway of interest. Van Doorsselaere *et al.* do not teach or suggest modifying the utilization of an intermediate substrate in a secondary metabolic pathway associated with a nutritional profile of a plant by introducing into the plant a nucleic acid molecule that encodes an enzyme not naturally occurring in the target secondary metabolic pathway as presently claimed.

Concerning 35 USC § 103

The Office Action contains rejections of the claims under 35 USC § 103 over various combinations of references. Applicants believe that the presently amended claims patentably distinguish from all of the cited references or any combination thereof. Below, Applicants address each of the

combinations of references cited by the Examiner, as they pertain to the presently amended claims.

EP 0 818 138 A1 to Murata in view of WO 96/00789 to Londesborough *et al.*

Murata describes transformation of *Arabidopsis thaliana* and rice with a gene encoding choline oxidase for the purpose of obtaining osmo-tolerant (drought-resistant) plants. The Examiner acknowledges at the last line of page 10 of the Office Action that Murata does not teach methods wherein the plant is not rice or *Arabidopsis*.

The Examiner then cites Londesborough *et al.* as teaching methods for the production of osmo-tolerant plants and as reciting a number of different plant species for which it would be desirable to produce osmo-tolerant versions.

The Examiner concludes that it would have been obvious to utilize the methodology of Murata on other plant species in order to provide drought-tolerant plants, because Murata teaches that a wide variety of plants can be conferred salt tolerance and/or osmotolerance, and Londesborough *et al.* particularly teach that this property would be of benefit for a wide range of plants.

The presently amended claims specify that the enzyme-coding sequence is operably linked to a seed-specific promoter. Neither Murata nor Londesborough *et al.* teach or suggest the use of a seed-specific promoter. Indeed, as discussed in previous amendments, the use of a seed-specific promoter would be contrary to Murata and Londesborough *et al.'s* objective of obtaining an osmo-tolerant plant, wherein it is necessary that most or all cells of the plant possess the property of osmo-tolerance, and not merely the seeds. Therefore, neither Murata nor Londesborough *et al.* teach or suggest the use of a seed-specific promoter as instantly claimed.

Murata in view of WO 92/01042 to Willmitzer *et al.*

The Examiner acknowledges that Murata does not teach methods in which the promoter is tissue selective or specifically seed selective, and Murata does not teach plants that are not rice or *Arabidopsis* (see the Office Action at page 13, second paragraph).

The Examiner looks to Willmitzer, *et al.* to cure these deficiencies in Murata. The Examiner contends that Willmitzer, *et al.* teach transgenic plants expressing industrial enzymes and that Willmitzer, *et al.* teach that the DNA sequence encoding the enzyme of interest may be under the control of a seed-specific promoter and that a variety of plants are useful for the introduction of the enzyme.

Although the Examiner describes this combination of references as a rejection over Murata in view of Willmitzer, *et al.*, Applicant's respectfully submit that the Examiner then actually argues the converse – i.e. Willmitzer, *et al.* in view of Murata. The Examiner argues that it would have been obvious to use seed-specific promoters for the expression of choline oxidase in plants as taught by Willmitzer, *et al.* The Examiner states that Willmitzer, *et al.* teach the industrial expression of enzymes in a wide range of plants and that Murata provides the nucleic acid sequence encoding choline oxidase and also that choline oxidase is a commercially available enzyme. Thus, the Examiner concludes that Willmitzer, *et al.* provide the necessary suggestion and direction to motivate the production of choline oxidases in plants, and thus, in the absence of secondary consideration such as unexpected results, concludes that the claimed invention is obvious over the prior art.

Applicants respectfully traverse this rejection.

First, with respect to the combination of Murata in view of Willmitzer, *et al.*, the modification of Murata (which concerns osmo-tolerance) to incorporate the use of seed-specific promoters would render Murata unsatisfactory for its intended purpose of conferring osmo-tolerance to the plant, for reasons discussed previously. Applicants respectfully submit that if

the proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. *In re Gordon* 733 F. 2d 900, 221 USPQ 1125 (Fed. Cir. 1984).

Concerning Willmitzer, *et al.* in view of Murata, which is not the rejection made by the Examiner, Willmitzer, *et al.* are concerned with the general concept of the industrial production of enzymes in plants (i.e. so-called "gene farming") and therefore naturally discusses a range of possible plant species that could be used as well as different promoters, such as seed-specific promoters. The Examiner takes the position that even though Willmitzer, *et al.* do not recite or suggest choline oxidase as a potential enzyme for industrial expression in plants, it would be obvious to do so merely because Murata provides that choline oxidase is a commercially-available enzyme that can be expressed in plants.

Applicants respectfully traverse this rejection and submit that the mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F. 2d 680, 16 USPQ 2d 1430 (Fed. Cir. 1990). There is nothing in Willmitzer, *et al.* or Murata to suggest the advantages discovered by the instant Applicants in transforming plants, especially crucifers, with choline oxidase, to reduce levels of the anti-nutritional compound sinapine. There is no teaching or recognition in the references that transformation of the plant with choline oxidase would function to modify the utilization of an intermediate substrate in a secondary metabolic pathway, as described and claimed by the instant Applicants.

The Examiner has acknowledged the importance of secondary considerations such as unexpected results. In the instant case, Applicants have demonstrated the surprising advantage of the transformation of plants, particularly crucifers, with choline oxidase, thereby modifying the utilization

of an intermediate substrate in the phenylpropanoid pathway, thereby reducing sinapine levels in the genetically altered plants.

As discussed in Applicants' previous submissions, Willmitzer, *et al.* take pains to distance themselves from enzymes that confer improved growth properties or physical characteristics on plants (see e.g., page 2, lines 23-28 of Willmitzer, *et al.*). The Examiner has noted that Willmitzer, *et al.* state, at page 3, lines 19-2,1 that any such improved desirable physical characteristics will be incidental to the true purpose of synthesizing the enzyme. This recognition that improved physical characteristics might possibly result is a far cry from suggesting their desirability. That Willmitzer *et al.* do not exclude potentially industrially useful enzymes does not teach or suggest the advantages of particular enzymes, such as choline oxidase, in modifying the utilization of an intermediate substrate in a secondary metabolic pathway, to confer an improved nutritional profile on the plant. Willmitzer *et al.*'s general teachings concerning the advantages of industrial-scale production of enzymes in plants does not suggest the desirability of the use of choline oxidase, when this is merely one of myriad possible enzymes that might be expressed in plants and Willmitzer, *et al.* had no recognition of the surprising advantages that expression of choline oxidase would provide. Indeed, as discussed above, Willmitzer, *et al.* took pains to divorce themselves from the possibility that expression of the enzyme would improve the physical properties of the plant itself!

Chapple *et al.* in view of both Kennley *et al.* (U.S. 5,662,958) and Willmitzer *et al.*

The Examiner cites Chapple *et al.* as above as teaching transformation of plants with the F5H gene in order to alter the lignin content in plants. The Examiner acknowledges that Chapple *et al.* do not teach use of a seed-selective promoter. The Examiner states that Kennley *et al.* teach that lignin within canola seeds prevents extensive degradation of cellulose and hemicellulose by celluloytic microorganisms (presumably to show that it would be desirable to modulate lignin content specifically in seeds) and that Willmitzer, *et al.*

teaches seed-directed expression of heterologous polypeptides in plants by placing the DNA sequence encoding the enzyme of interest under the control of a seed-specific promoter.

Applicants respectfully submit that the presently amended claims patentably distinguish from Chapple *et al.* in view of Kennley *et al.* or Willmitzer, *et al.*

As discussed above, the F5H enzyme is an enzyme within the portion of the phenylpropanoid metabolic pathway that relates to a lignin synthesis (see e.g. Figure 1 of Chapple *et al.*). The presently amended claims specify that the enzyme does not naturally occur in the secondary metabolic pathway in which the utilization of an intermediate substrate is to be modified. Chapple *et al.* do not teach or suggest transforming a plant cell with a nucleic acid sequence encoding an enzyme capable of modifying the utilization of an intermediate substrate in a secondary metabolic pathway associated with a nutritional profile of a plant, wherein the enzyme does not naturally occur in the secondary metabolic pathway. Neither Kennley *et al.* nor Willmitzer, *et al.* cure this deficiency in the teaching of Chapple *et al.*

Van Doorsselaere *et al.* in view of Chapple *et al.* (The Plant Cell, Volume 4, 1413-1424)

The Examiner cites Van Doorsselaere *et al.* for the reasons discussed previously herein. Specifically, the Examiner states that Van Doorsselaere *et al.* teach the transformation of plants with a nucleic acid encoding O-methyl transferase (OMT) in order to alter the lignin content in plants. But the Examiner acknowledges that Van Doorsselaere *et al.* do not teach a method in which the transgenic plants are assayed for sinapine content.

Applicants respectfully submit that the presently amended claims patentably distinguish from Van Doorsselaere *et al.* in view of the Chapple *et al.* As discussed above, the OMT enzyme described by Van Doorsselaere *et al.*, like the F5H enzyme described by Chapple *et al.*, naturally occurs within the portion of the phenylpropanoid secondary metabolic pathway that concerns

lignin biosynthesis. This is shown clearly in Figure 1 of the cited Chapple *et al.* article in The Plant Cell, Volume 4, pages 1413-1424. Thus, Van Doorsselaere *et al.* do not teach or suggest transforming a plant cell with a nucleic acid sequence that encodes an enzyme capable of modifying utilization of an intermediate substrate in a secondary metabolic pathway associated with a nutritional profile of a plant, wherein the enzyme does not naturally occur in the secondary metabolic pathway, as presently claimed. Chapple *et al.* does not cure this deficiency in the Van Doorsselaere *et al.* reference.

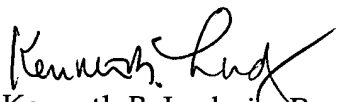
Concerning Allowable Subject Matter

Applicants acknowledge the Examiner's finding that the prior art does not teach or suggest methods in which both choline oxidase and betaine aldehyde dehydrogenase are introduced into the same plant under the control of a seed-specific promoter. New claims 85 and 93 are directed to this subject matter.

In view of all the foregoing, entry of the amendments and further consideration of this application, leading to its timely Allowance, are respectfully requested.

If it is believed that a telephone conference with Applicants' attorney would expedite the prosecution of this application, the Examiner is requested to call the undersigned at the below-identified telephone number.

Respectfully submitted,



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Version With Markings to Show Changes Made

Claims 34-38, 40-46 and 48-80 have been cancelled.

Claims 81-98 are new.